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Marketing Research Report No. 882

U.S. DEPT. OF AGRICULTURE
Agricultural Research Service

MAR 31 1971

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Preliminary Evaluation of New Candidate Materials as Toxicants, Repellents, and Attractants Against Stored-Product Insects—I

Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

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PREFACE

This publication reports the results of a series of tests to evaluate the effectiveness of selected insecticides against stored-product insects. This study is part of a broad program of continuing research, and results of similar studies will be published as the tests are completed. Those compounds that show promise in the study reported here will be tested further in field trials.

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CAUTION: Pesticides can be injurious to humans, domestic animals, desirable plants, and fish or other wildlife—if they are not handled or applied properly. Use all pesticides selectively and carefully. Follow recommended practices for the disposal of surplus pesticides and pesticide containers.



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Washington, D.C.

Issued August 1970

For sale by the Superintendent of Documents, U.S. Government Printing Office
Washington, D.C. 20402 - Price 15 cents

Preliminary Evaluation of New Candidate Materials as Toxicants, Repellents, and Attractants Against Stored-Product Insects—I

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SUMMARY

Preliminary evaluation of new promising chemicals for toxicity and repellency were conducted at the Stored-Product Insects Research and Development Laboratory, Savannah, Ga. Six compounds were tested against adult confused flour beetles, *Tribolium confusum* Jacquelin duVal, and black carpet beetle larvae, *Attagenus megatoma* (F.). Of the six compounds tested, four showed promise as direct-contact toxicants against

both species. These four were Bay 75546 (ENT-27607), Bay 79845 (ENT-27608), Bay 88991 (ENT-27626), and Penick SBP1382 (ENT-27474). All of the compounds, except Bay 88991, when tested as residuals, were generally less toxic to the insects than was the malathion standard. Two compounds, Bay 88991 and Penick SBP1382, showed promise as repellents against confused flour beetles.

INTRODUCTION

The preliminary evaluation of experimental compounds to determine their efficacy as direct-contact, residue, and vapor toxicants and as repellents and attractants is a continuing phase of the research conducted at the Stored-Product Insects Research and Development Laboratory, Savannah, Ga. The standard test methods

for direct-contact and residue toxicity and repellency are described in detail in this report. They will be referred to in all future reports in this series.

This paper gives the results of preliminary laboratory direct-contact and residue toxicity tests and repellency tests with the following experimental compounds:

Company designation ¹	ENT number	Chemical name	Mammalian toxicity per kilogram (acute oral, rats)
Chemagro: Trichlorfon	19763	dimethyl(2,2,2-trichloro-1-hydroxyethyl) phosphonate	Mg. 560-630
Geigy: GS-19849	27605	ethyl 4,4'-dibromobenzilate	ca. 5,000
Penick: SBP1382	27474	(5-benzyl-3-furyl)methyl 2,2-dimethyl-3-(2-methylpropenyl)cyclopropane-carboxylate	> 1,500

See footnote at end of tabulation.

¹ Agricultural research technicians and research entomologist, respectively, Stored-Product Insects Research and Development Laboratory, Savannah, Ga.

Company designation ¹	ENT number	Chemical name	Mammalian toxicity per kilogram (acute oral, rats)
Vero Beach:			<u>Mg.</u>
Bay 75546	27607	0-(3-bromo-5,7-dimethylpyrazolo-[1,5-a] pyrimidin-2-yl) 0,0-diethyl phosphorothioate	1,000
Bay 79845	27608	0-(3-chloro-5,7-dimethylpyrazolo-[1,5-a] pyrimidin-2-yl) 0,0-diethyl phosphorothioate	>2,500
Bay 88991	27626	diethyl phosphate ester with o-tolylglyoxylonitrile oxime	>1,000
American Cyanamid:			
Malathion	17034	S-[1,2-bis(ethoxycarbonyl)-ethyl]=0,0-dimethyl phosphorodithioate	1,000-1,375

¹These compounds and their names and mammalian toxicity values were received through Pesticide Chemicals Research Branch, Entomology Research Division, Agricultural Research Service, Beltsville, Md.

Information previously obtained from this phase of research has been published by the Department in Marketing Research Reports 324 and 546.²

PROCEDURE

Test Insects

The insects used in the tests were confused flour beetle adults, *Tribolium confusum* Jacquelin duVal, 7 to 14 days old, and black carpet beetle larvae, *Attagenus megatoma* (F.), 3 to 5 months old.

The confused flour beetles were reared in a 50:50 mixture of white flour and cornmeal and the black carpet beetles in Purina laboratory chow meal. These media also contained 5 percent of brewer's yeast. The insects were reared in a room in which the temperature was maintained at $27^{\circ} \pm 1^{\circ}$ C. and the relative humidity at 60 \pm 5 percent. All testing, post exposure, and aging of residues were performed under similar conditions.

Exposure Methods

Standard Method Number 1 (Direct-Contact Toxicity)

The candidate materials were prepared as acetone solutions at concentrations calculated so that 0.5 micro-liter (μ l.) would result in dosages of 0.4, 0.8, and 1.6 micrograms (μ g.) per insect when applied topically. The dosage 1.6- μ g. per insect was tested first. If the new material was effective at this rate of application (that is,

killed 50 percent or more of the insects), the two lower dosages were also tested. If the new material was not effective at 1.6 μ g. per insect, no further studies were conducted under this method. Past experience has shown that eliminating chemicals from further testing on such a basis does not exclude those that may show promise on complete testing or those that may be of special interest because of low mammalian toxicity.

Confused flour beetle adults and black carpet beetle larvae were used in this method. The insects were anesthetized with carbon dioxide in a Buchner funnel (for periods not exceeding 5 minutes).³ The immobilized insects were then individually held with a small suction tube and the insecticide was applied to the

²U.S. Agricultural Research Service, Stored-Product Insects Laboratory. Laboratory evaluation of promising compounds as repellents to flour beetles, *Tribolium* spp. U.S. Dept. Agr. Market. Res. Rpt. 324, 46 pp. 1959, and Supplement 1, 46 pp. 1964; and Speirs, Roy D. Contact, residue, and vapor toxicity of new insecticides to stored-product insects. U.S. Dept. Agr. Market. Res. Rpt. 546, 31 pp. 1962.

³Longer exposure periods have shown an increase in the effectiveness of malathion against these two insect species. Young, Seth Y., III, and McDonald, Lehman L. Effect of CO₂ anesthesia on malathion toxicity to four species of stored-product insects. Ann. Ent. Soc. Amer. 63(2): 381-382. 1970.

dorsal surface of their thorax with an automatic micro-applicator. Eighty insects of each species were treated at each dosage level. In addition, the same number were treated with acetone as controls. Malathion was used as the standard for comparison.

After the insects were treated, they were placed in clean containers and held for observation. The toxicities of the insecticides were determined by the number of insects knocked down or dead-plus-moribund observed 120 hours after application for adult flour beetles and 336 hours after application for black carpet beetle larvae. Insects that were not able to run about were recorded as knocked down. Of these insects, those that did not move or that responded only feebly when probed lightly were recorded as dead-plus-moribund.

Standard Method Number 2 (Residue Toxicity)

The experimental insecticides were prepared in acetone solutions and applied to 3- by 12-in. strips of aluminum foil laminated to 40-lb. kraft paper with a Gardner automatic blade applicator. The insecticides were first tried in an exploratory test as 1-day-old residues at rates of application of 50 $\mu\text{g}/\text{cm}^2$ on the aluminum surfaces and 100 $\mu\text{g}/\text{cm}^2$ on the paper surfaces. Insecticides that were not effective were not tested further. If a chemical killed 50 percent or more of the insects on either surface, it was tested further; however, if less than 50-percent kill was obtained on either surface, that surface was not tested further.

Four open-end glass cylinders, 6.4 cm. in diameter and 2.5 cm. in height, were placed on each treated surface. Ten confused flour beetles were placed in each cylinder and exposed 4 hours. Ten black carpet beetle larvae were placed in each cylinder on other treated strips and exposed 24 hours. The insects were then transferred to clean petri dishes for post-exposure observations. The number of knocked down and dead-plus-moribund insects was recorded 120 hours after exposure for flour beetles and 168 hours after exposure for black carpet beetle larvae.

If the insecticide showed promise in the exploratory test, it was further tested by these same procedures at lower rates of 5, 10, and 50 $\mu\text{g}/\text{cm}^2$ on paper surfaces and 1, 5, and 25 $\mu\text{g}/\text{cm}^2$ on aluminum surfaces as 1-day-old residues. It was also tested as 28-day-old residues at rates of application of 10, 50, and 100 $\mu\text{g}/\text{cm}^2$ on paper surfaces and 50 $\mu\text{g}/\text{cm}^2$ on aluminum surfaces. The flour beetles were exposed for 24 hours to the 28-day-old residues; otherwise, these tests were conducted the same as those for the 1-day-old

residues. Malathion-treated surfaces were used as standards for comparison, and acetone-treated surfaces were used as controls.

Standard Method Number 3 (Repellency or Attractancy)

Strips of aluminum foil, laminated to 40-lb. kraft paper, 4 by 16 in. were treated on the paper side with acetone solutions of the candidate repellent. The solutions were applied with a Gardner automatic blade applicator at rates of 25, 100, and 200 $\mu\text{g}/\text{cm}^2$. Strips treated with pyrethrins at 5 $\mu\text{g}/\text{cm}^2$ in combination with piperonyl butoxide at 50 $\mu\text{g}/\text{cm}^2$ were used as standards for comparison. Strips treated with pyrethrins at 5 $\mu\text{g}/\text{cm}^2$ and others with piperonyl butoxide at 50 $\mu\text{g}/\text{cm}^2$ were also included for comparison.

In these tests, 8-in. strips of treated and untreated paper were joined edge-to-edge lengthwise with cellulose tape on the untreated side. Two such test surfaces were positioned so that the treated half of one was turned to the right and the treated half of the other turned to the left to counteract any undetermined external influence on the distribution of the test insects. Two glass cylinders, 2.5 cm. in height and 6.4 cm. in inside diameter, were placed on each of the two sections of paper to provide test arenas of equal areas of treated and untreated paper. Two sets (four arenas each) of untreated paper matched with untreated paper were used as checks.

Ten confused flour beetle adults were exposed in each test arena, and the number of insects on the treated half and on the untreated half of the arena was recorded at 9 a.m. and 3 p.m. After application of the chemicals, exposures to determine the average numbers of insects on the untreated half of the repellency arena during a 5-day period was initiated at 4 days, 2 weeks, 1 month, and 2 months. The averages were converted to express "percent repellency or attractancy" by doubling the difference between the percentage of insects counted on the untreated half and the 50-percent distribution expected if only untreated papers were used. Positive figures (+) expressed repellency and negative figures (-) attractancy. These were listed under six classes of repellency as follows:

Class	Repellency (percent)
0	>-0.1 to <0.1
I1 to 20
II	20.1 to 40
III	40.1 to 60
IV	60.1 to 80
V	80.1 to 100

The same criteria were used for attractancy except that the repellency percentages were all negative. Class III was generally regarded as the minimum repellency for further consideration; however, the main selection factor

was whether the chemical was more repellent than the pyrethrins-piperonyl butoxide standard or not. A generally accepted standard for attractancy has not yet been established.

RESULTS AND DISCUSSION

As direct-contact toxicants, Bay 75546, Bay 79845, and Bay 88991 were more effective against black carpet beetle larvae than malathion (table 1). The toxicity of Penick SBP1382 was comparable to that of malathion against black carpet beetle larvae. Bay 88991 was only slightly less toxic than malathion to confused flour beetle adults. The other compounds were less toxic than malathion to the test insects. The mammalian toxicities of the four most effective compounds are similar to or less than that of malathion (see tabulation on p. 1). This is an additional factor contributing to their potential as suitable materials for control of stored-product insects.

Geigy GS-19849 and trichlorfon were not effective in the exploratory tests, so they were eliminated from further residue tests. Bay compounds 75546, 79845, and 88991 and Penick SBP1382 were included in the complete residue tests without first conducting the exploratory test. Residues of all of these test compounds were generally less effective than malathion residues against confused flour beetle adults and black carpet beetle larvae (tables 2 and 3). However, Bay 88991 was the most toxic of the candidate insecticides to black carpet beetle larvae. As residues on aluminum surfaces, Bay 88991 was similar to malathion to black carpet beetle larvae initially and more toxic than malathion residues after aging for 28 days. Its residues on paper surfaces were somewhat less toxic than those of malathion.

Bay 75546 and Bay 79845 were similar to malathion in toxicity to black carpet beetle larvae as aged residues on aluminum surfaces. The only compound that demonstrated appreciable residue toxicity to confused flour beetles was Bay 88991. It was generally less effective than malathion; however, as aged residues on aluminum surfaces, Bay 88991 indicated higher toxicity to the insects than did malathion.

Bay 88991 was too toxic to be tested at the application level of 200 $\mu\text{g}/\text{cm}^2$ and could not be evaluated at the application level of 100 $\mu\text{g}/\text{cm}^2$ until the treatment had aged for 2 months. The percentage of repellency at the 2-month aging test for this level was 28.0 as compared with a percentage repellency of 28.1 for the standard at the same testing period (table 4). One compound, SBP1382, was more repellent to the test insects, *T. confusum* adults, than was the standard. It showed an average percentage repellency of 50.3 (Class III) at the 25 $\mu\text{g}/\text{cm}^2$ rate of application. This compound was too toxic to the test insects to be evaluated at the higher application levels. The pyrethrins-piperonyl butoxide standard gave an average percentage repellency of 37.4 (Class II). None of the other compounds tested produced any appreciable repellencies.

None of the compounds showed potential as attractants.

Table 1.—*Toxicity of 7 compounds applied topically to adult confused flour beetles and black carpet beetle larvae at 0.5 µl. of acetone solution per insect*

Compound	Rate of application per insect	Effect of compound on—			
		Adult flour beetles 120 hours after exposure		Black carpet beetle larvae 336 hours after exposure	
		KD	D+M	KD	D+M
Chemagro: Trichlorfon	µg. 1.6	Pct. 0	Pct. 0	Pct. 18	Pct. 15
Geigy: GS-19849	1.6	0	0	0	0
Penick: SBP13824 .8 1.6	35 58 98	30 53 92	95 100 100	50 75 95
Vero Beach: Bay 755464 .8 1.6	20 52 65	20 50 65	100 100 100	100 100 100
Bay 798454 .8 1.6	62 80 92	62 80 92	100 100 100	90 95 100
Bay 889914 .8 1.6	75 92 100	52 92 100	100 100 100	100 100 100
American Cyanamid: Malathion.4 .8 1.6	98 100 100	98 100 100	98 100 100	75 85 85
None (solvent)	--	0	0	0	0

Note—KD = knockdown; D+M = dead-plus-moribund.

Table 2.—*Residue toxicity of 5 compounds to adult confused flour beetles exposed 4 hours and black carpet beetle larvae exposed 24 hours to 1-day-old treated surfaces*

Surface and compound	Rate of application	Effect of compound on—			
		Adult flour beetles 120 hours after exposure		Black carpet beetle larvae 168 hours after exposure	
		KD	D+M	KD	D+M
<i>Paper surface</i>					
Penick:	μg./cm. ²	Pct.	Pct.	Pct.	Pct.
SBP1382	5	0	0	0	0
	10	0	0	0	0
	50	0	0	8	2
Vero Beach:					
Bay 75546	5	2	2	12	10
	10	0	0	18	10
	50	0	0	0	0
Bay 79845	5	0	0	82	20
	10	0	0	60	25
	50	0	0	25	10
Bay 88991	5	0	0	10	5
	10	0	0	100	25
	50	0	0	100	32
American Cyanamid:					
Malathion	5	8	8	100	20
	10	65	62	100	32
	50	100	95	100	55
None (solvent)	--	0	0	0	0
<i>Aluminum surface</i>					
Penick:					
SBP1382	1	0	0	0	0
	5	0	0	0	0
	25	5	5	22	2
Vero Beach:					
Bay 75546	1	0	0	0	0
	5	2	2	0	0
	25	2	2	0	0
Bay 79845	1	0	0	2	0
	5	0	0	0	0
	25	0	0	85	18
Bay 88991	1	0	0	100	35
	5	5	5	100	48
	25	98	98	100	50
American Cyanamid:					
Malathion	1	55	55	80	20
	5	100	100	100	50
	25	100	100	100	52
None (solvent)	--	0	0	0	0

Note.—KD = knockdown; D+M = dead-plus-moribund.

Table 3.—*Residue toxicity of 5 compounds to adult confused flour beetles and black carpet beetle larvae exposed 24 hours to 28-day-old treated surfaces*

Surface and compound	Rate of application	Effect of compound on—			
		Adult flour beetles 120 hours after exposure		Black carpet beetle larvae 168 hours after exposure	
		KD	D+M	KD	D+M
<i>Paper surface</i>					
Penick:	μg./cm. ²	Pct.	Pct.	Pct.	Pct.
SBP1382	10	0	0	0	0
	50	0	0	0	0
	100	0	0	0	0
Vero Beach:					
Bay 75546	10	0	0	24	8
	50	0	0	5	2
	100	0	0	75	59
Bay 79845	10	0	0	62	39
	50	0	0	15	9
	100	0	0	20	15
Bay 88991	10	0	0	2	2
	50	2	2	98	75
	100	48	48	100	90
American Cyanamid:					
Malathion	10	85	85	55	10
	50	100	100	100	45
	100	100	100	100	55
None (solvent)	--	0	0	0	0
<i>Aluminum surface</i>					
Penick:					
SBP1382	5	0	0	0	0
	25	0	0	0	0
	50	0	0	0	0
Vero Beach:					
Bay 75546	5	0	0	25	23
	25	15	15	98	68
	50	26	26	97	57
Bay 79845	5	2	2	42	22
	25	10	10	95	81
	50	16	12	100	53
Bay 88991	5	8	8	65	42
	25	100	100	100	95
	50	100	100	100	90
American Cyanamid:					
Malathion	5	0	0	0	0
	25	100	100	100	60
	50	100	100	100	70
None (solvent)	--	0	0	0	0

Note.—KD = knockdown; D+M = dead-plus-moribund.

Table 4.—*Repellency of 6 candidate compounds to adult confused flour beetles*

Compound	Rate of application	Repellency at indicated age of residues					Repellency class ¹
		1 wk.	2 wk.	1 mo.	2 mo.	Average	
Chemagro:	μg./cm. ²		Pct.	Pct.	Pct.	Pct.	
Trichlorfon	25	5.0	(2)	7.0	-19.4	-2.5	IA
	100	11.6	(2)	0	31.8	14.5	I
	200	13.0	(2)	15.0	9.4	12.5	I
	400	20.0	(2)	25.4	13.8	19.7	I
Geigy:							
GS-19849.	25	14.4	-8.6	19.0	18.6	10.9	I
	100	13.2	13.6	24.6	27.6	19.8	I
	200	10.6	11.0	30.4	17.0	17.3	I
Penick:							
SBP1382	25	66.0	41.8	44.6	48.6	50.3	III
	³ 100	--	--	--	--	--	--
	³ 200	--	--	--	--	--	--
Vero Beach:							
Bay 75546	25	-7.6	0.6	11.4	15.6	5.0	I
	100	-4.2	4.2	10.0	-6.4	0.9	I
	200	-21.0	1.4	12.0	17.4	2.5	I
Bay 79845	25	8.6	2.6	8.8	14.6	8.7	I
	100	-6.0	-1.0	10.0	9.0	3.0	I
	200	15.6	9.6	32.8	18.4	19.1	I
Bay 88991	25	3.6	-9.0	23.8	22.4	10.2	I
	100	(3)	(3)	(3)	28.0	⁴ 28.0	II
	³ 200	--	--	--	--	--	--
FMC:							
Pyrethrins ⁵	5	26.5	5.2	16.6	11.9	15.1	I
Piperonyl butoxide ⁵ . . .	50	27.4	12.0	26.4	29.7	23.9	II
Standard ⁵	5	57.3	28.1	36.2	28.1	37.4	II
None (untreated) ⁵	--	±5.5	±5.6	±10.9	±17.2	±9.8	--

¹Numbers followed by A are attractancy class values.²No results because of sickly condition of test insects.³Toxicity to the test insects too high for testing repellency.⁴Results of only the 2-month aging test (not an average).⁵Average of data from 3 experiments.